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Square Arrays in Early Cortical Lens Opacities

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A combined freeze-fracture and scanning electron microscopic study of early opaque spots in the aging human lens showed the absence of gap junctions and the presence of square arrays in the membranes of disturbed fibers and neighboring unaffected fibers. Square arrays, with membrane particles of 6–7 nm, are considered as rearranged gap junctions and/or intramembranous particles, with particle sizes between 8.5–9.5 nm; they are a sign of electric and metabolic uncoupling. These ultrastructural observations lend support to the idea of an uncoupling mechanism in the aging human lens, conserving the transparency of unaffected parts of the lens, as postulated previously. Invest Ophthalmol Vis Sci 31:2476–2481, 1990

As reemphasized by Bron and Brown,[1] most nuclear cataracts must be ascribed to increased light scattering due to increased amounts of high-molecular-weight proteins, and most cortical cataracts are the result of disturbance of the integrity of lens fibers. Cortical cataract is the most prevalent age-related type of cataract.[2,3] Since cortical opacities in early stages tend to be stationary and as it is well established that lens fibers are extensively coupled by gap or communicating junctions,[4] Bron and Brown[1] postulated a mechanism in the lens which "conserves the transparency of unaffected parts of the lens." (p. 4) In

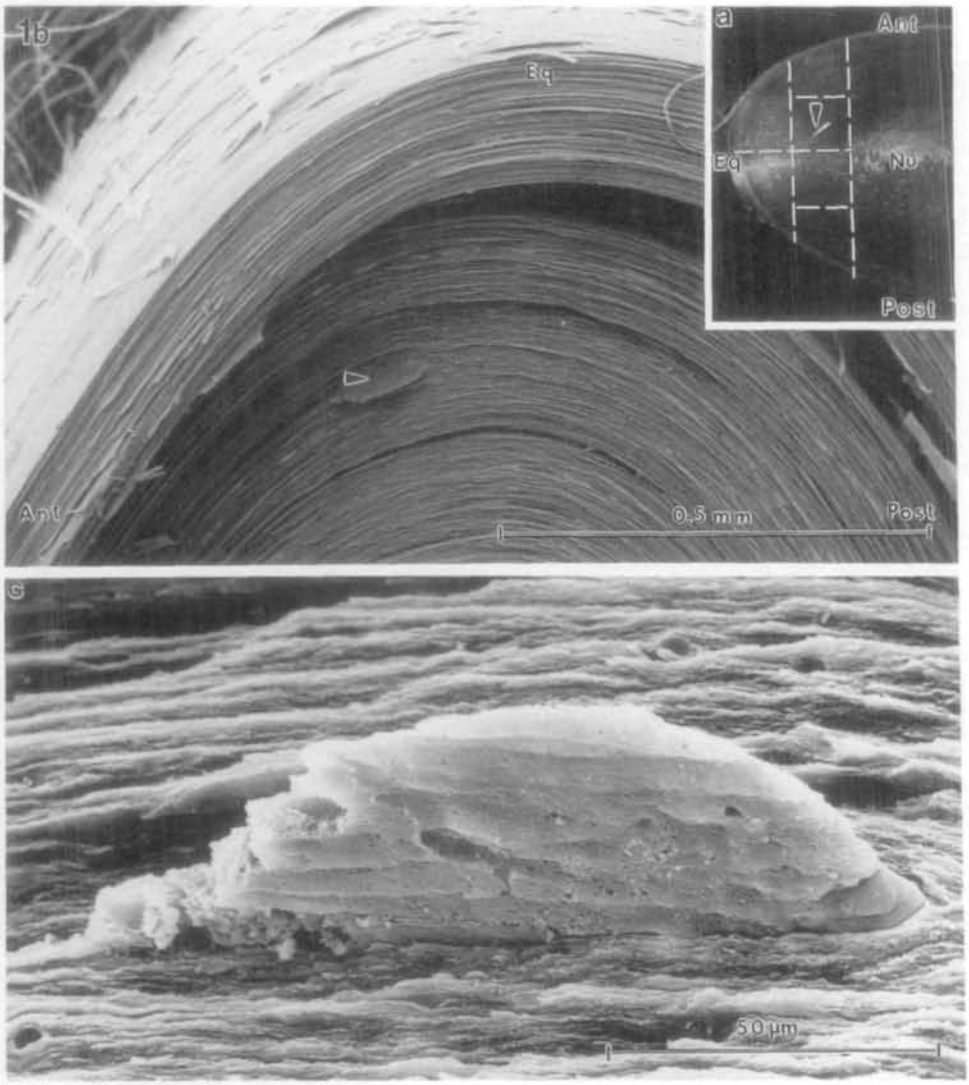

Fig. 1. Panel showing the sampling procedure used in this study (**A**) and exemplifying the dark field stereomicroscopic (**A**; arrowhead), the low power SEM (**B**; arrowhead), and medium power SEM (**C**) appearance of radial shades as studied in this paper. (**A**) An axial slice (thickness 1–2 mm) from which small pieces (dashed lines) are dissected for freeze fracture. The white scatter in the equator and nucleus is an artefact of the slice cutting. The SEM pictures clearly show the position of the radial shade within the lens. They also indicate that radial shades are mechanically weakly connected to neighboring normal fibers and are easily fractured apart. Eq = equator; Post = posterior cortex; Ant = anterior cortex; Nuc = lens nucleus.

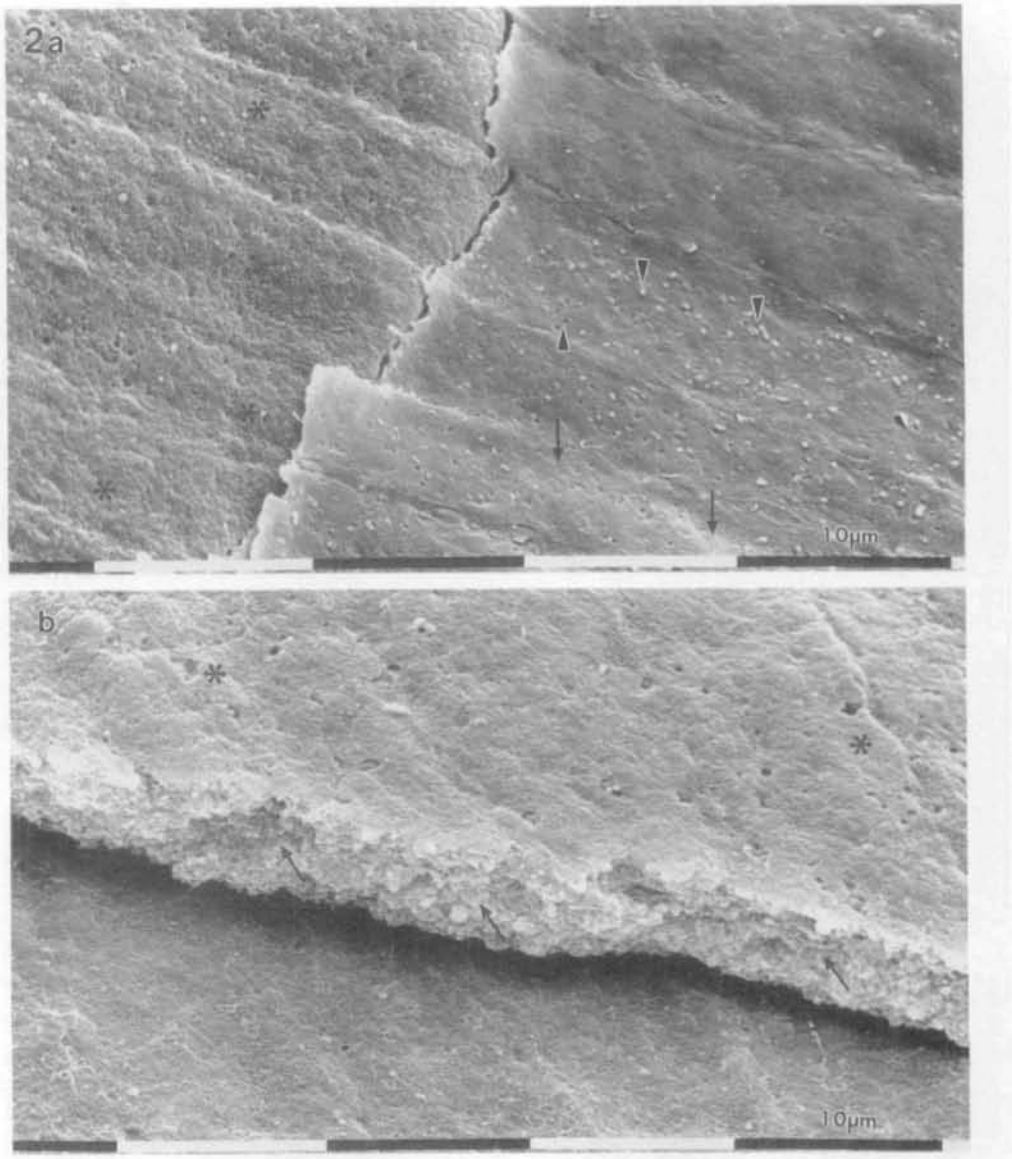

Fig. 2. SEM micrographs illustrating the discontinuity of the membranes of affected and unaffected parts of individual fibers (**A**) and the filling of the opaque fibers with vacuoles (**B**, arrows). Note in both micrographs the fine globular aspect of the affected membranes.* Figure 2**A** also exhibits the grooves-and-ridges (arrows) and balls-and-sockets (arrowheads) on the nonaffected membranes. The replicating of the fine globular aspect on adjacent nonaffected fibers is suggested in the lower part of Figure 2**B**.

a previous scanning electron microscopic (SEM) study of early opacities in the aging human lens,[5] it was seen that in radial shades or opaque spots the affected parts of individual fibers were separated from the unaffected parts and from the undisturbed neighboring fibers by membranes with a deviating ultrastructure. However, the restricted resolution of this SEM procedure did not allow conclusions to be drawn about the precise nature of this deviating membrane ultrastructure. In the course of a freeze-fracture study on the topographic variation of intramembranous particles (IMPs) and gap junctions (GJs) in the human lens, we accidentally fractured through radial shades,[5] unraveling the precise nature of the separating membranes.

Materials and Methods. Human lenses were obtained from donor eyes obtained for corneal transplantation and admitted to the Corneabank Amsterdam. After biomicroscopic screening, the lenses were fixed in a 0.08 M cacodylate buffered solution of 1.25% glutaraldehyde and 1.0% paraformaldehyde, pH 7.3. After fixation for several days to weeks, the lenses were dissected into small pieces (Fig. 1A). For SEM the pieces were rinsed in cacodylate buffer, dehydrated in a graded series of ethanol, critical point dried with CO_2, and coated with gold. For freeze fracture the pieces were rinsed in cacodylate buffer and infiltrated with 2.3 M sucrose for cryoprotection. The pieces were quick frozen in liquid propane and fractured in a Balzers BAF 300 (Balzers SA, Liechtenstein) at 160°K and 10^{-6} Pa. The fractures were coated with platinum and carbon and cleaned with perchloric acid; the replicas were studied in Philips EM 400 and 420 (Philips Industries, Eindhove, The Netherlands) transmission electron microscopes.

A sample of 20 largely clear lenses, from patients

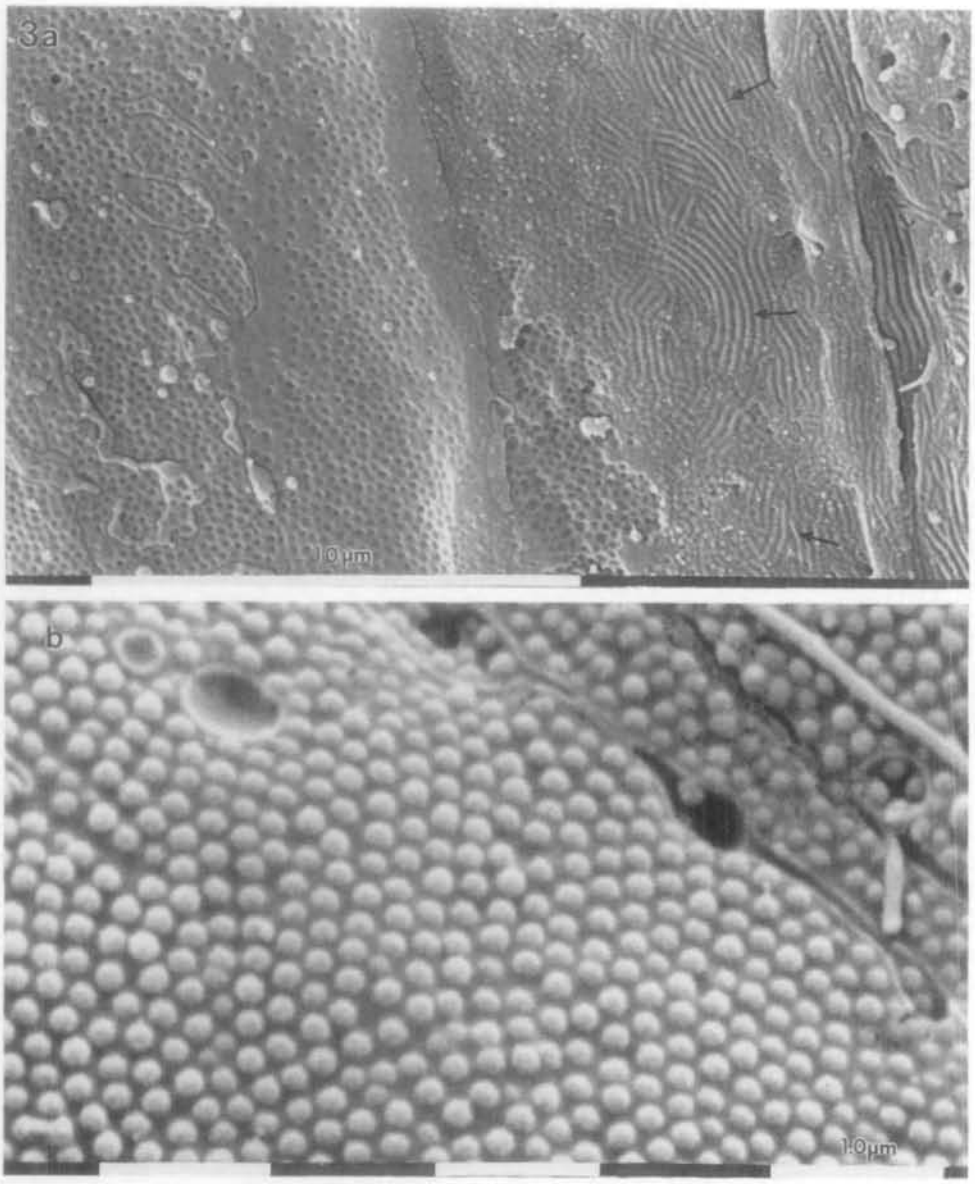

Fig. 3. Medium (**A**) and high power (**B**) SEM views of affected membranes illustrating the relation between the fine globular elements and the grooves-and-ridges (**A**; arrows) and the regular organization and rather uniform dimensions (130–170 nm) of the fine globular elements (**B**) (compare with Figure 5).

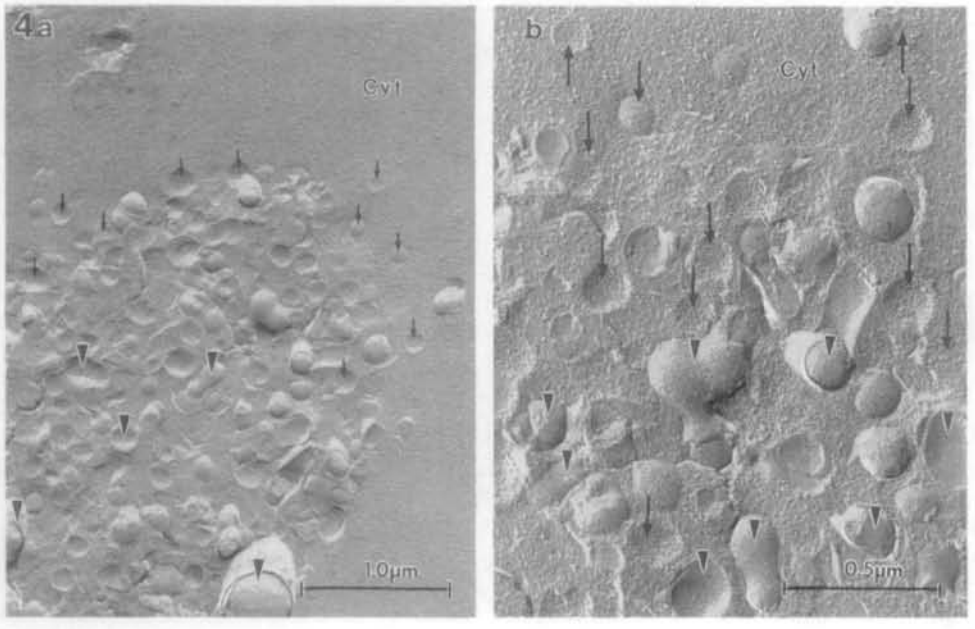

Fig. 4. Freeze fracture micrographs at low and medium power exhibiting the membranes at the border between the affected and nonaffected parts of an individual fiber. The affected part is filled with smooth membrane-bound vacuoles (arrowheads, **A** and **B**). In the border zone numerous particle-studded membranes are present (arrows, **A** and **B**). The paracrystalline organization of these particles is best appreciated at high magnification (**B**). Cyt = cytoplasm.

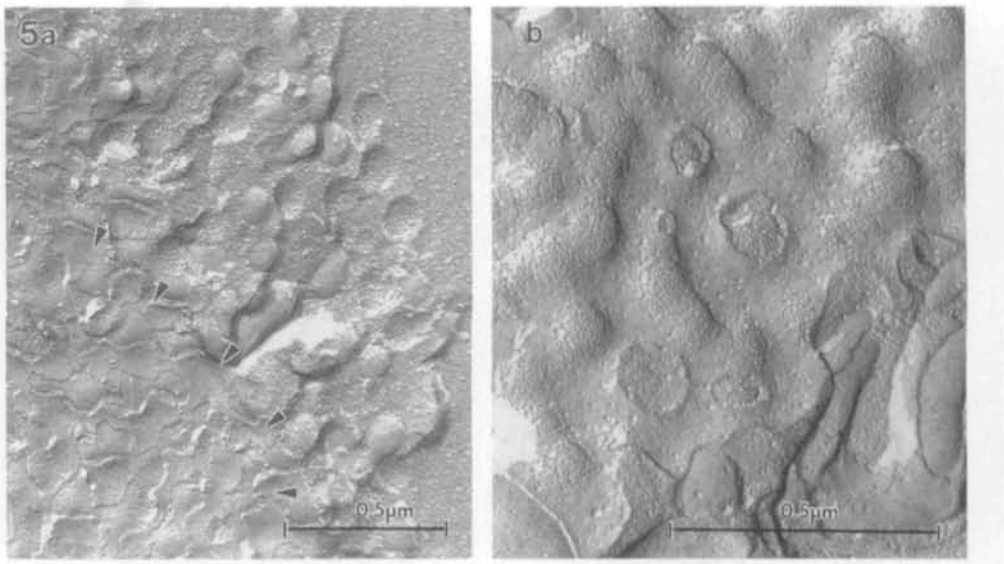

Fig. 5. Freeze fracture micrographs at the border between affected and nonaffected fibers more or less parallel to the surface (**A**). Note the particle-studded undulating membrane (arrowheads). At high magnification (**B**) the paracrystalline organization of the particles and the linear arrangement of these square arrays are evident. These square arrays have dimensions between 130 and 170 nm identical to the globular elements indicated in Figure 3**B**.

Fig. 6. Freeze fracture micrograph of a lens fiber membrane proximal to a disturbed region (**A**) showing elevated circular membrane fragments studded with paracrystalline arranged membrane particles reminiscent of square arrays (SA) on one side and free IMPs and GJs on the other side. The membrane particles of the SAs measure between 6 and 7 nm and the free IMPs and IMPs forming the GJs between 8.5 and 9.5 nm. To appreciate the difference between GJs and SAs, Figure 6**B** illustrates part of an unaffected lens fiber at an identical location in the lens and at a comparable magnification. Note the large and small GJs with their noncrystalline, random aggregation of particles (8.5–9.5 nm). Cyt = cytoplasm.

varying in age from premature to 86 years were used for the study of the topographic variation in membrane architecture. Four of these lenses contained small spot-like opacities or radial shades (Fig. 1). We investigated the fractures through the radial shades in detail.

Results. Low-power SEM views of radial shades (Fig. 1) showed that these opacities were entities which, on fracturing for SEM, tended to be isolated from the undisturbed fibers. In addition at medium-power magnification, the affected parts of individual fibers were discontinuous with their unaffected parts. The membranes of the affected parts had a fine, globular aspect, and the unaffected membranes had a normal aspect with grooves-and-ridges and balls-and-sockets (Fig. 2A). In cross-fracture (Fig. 2B) the opaque spots were filled with vacuoles of varying sizes and lined by a fine, globular membrane which was replicated on the membranes of neighboring unaffected fibers. At medium power (Fig. 3A) it became evident that, at the lateral border of a radial shade, the fine, globular elements of the unaffected part of a membrane were continuous with the grooves-and-ridges of the relative normal part of the membrane. At high magnification (Fig. 3B) the regularity of the globular aspect was seen with dimensions between 130–170 nm. Freeze-fracture observations of the border zones between opaque and clear zones in an individual fiber (Fig. 4) showed that the affected parts were filled with smooth membrane-bound vacuoles of varying sizes intermingling with a more uniform population of circular membranes studded with IMPs. These, at high magnification, had a paracrystalline organization reminiscent of square arrays (Fig. 4B). In a fracture beyond the fiber surface (Fig. 3A), the continuity of the IMP-studded membranous elements with the grooves-and-ridges of the relative normal part of the membrane became evident (Fig. 5A). At high magnification the paracrystalline organization of these membrane particles was observed as was their possible origin from grooves (Fig. 5B). No GJs were found on the affected membranes or the membranes in the direct border zones, but in remote sites on the same fiber, normal GJs were present (Fig. 6). The size of the circular membranes studded with paracrystalline membrane particles varied between 130–170 nm and was of the same order of magnitude as the globular elements found in SEM (Fig. 3B). When measured in the same fracture, isolated IMPs and IMPs forming the GJs had dimensions between 8.5–9.5 nm, but the particles forming the square arrays measured between 6–7 nm.

Discussion. Our main conclusion from this study is that, in addition to an aberrant SEM ultrastructure of the membranes of affected fibers and their neighboring fibers, these membranes are characterized by the absence of GJs and the presence of square arrays. The square arrays, characterized by a paracrystalline organization of their constituting membrane particles, are mainly in the form of circular membrane elevations. The size of these circular membrane elevations is in the same range as that of the globular membrane elements found in SEM (130–170 nm). In addition the membrane particles forming (1) the square arrays and (2) the free IMPs and IMPs in GJs are different sizes: 6–7 nm and 8.5–9.5 nm, respectively.

There is overwhelming evidence that GJs are communicating junctions,[6] responsible for the electric and metabolic coupling in the lens.[4] Under experimental conditions GJs and IMPs can crystallize and form square arrays.[7,8] In the lens this leads to uncoupling of the fibers involved.[6] Our observation of square-array formation in membranes of opaque fibers and neighboring unaffected fibers suggests a similar process of uncoupling in the aging human lens. This agrees with observations in an inherited cataract in mice[9] and in advanced human cataracts.[10]

As reported previously[5] early opacities are segregated from neighboring undisturbed fibers; within an individual fiber, they are sealed off from the unaffected part by membranes with an abberrant SEM ultrastructure. The absence of GJs and the presence of square arrays on these disturbed membranes further substantiate this observation. Our study lends support to the idea that these aberrant membranes are responsible for the uncoupling of affected from unaffected fibers as postulated by Bron and Brown[1] to explain the stationary or slowly progressive character of early equatorial cortical opacities.

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